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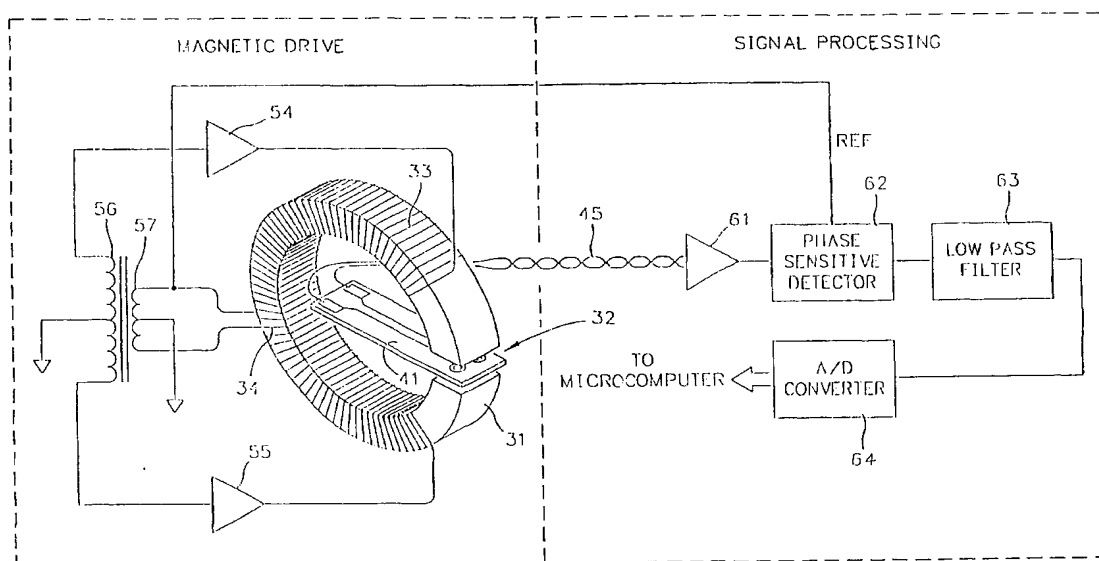
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(54) Title: METHOD AND APPARATUS FOR MAKING MEASUREMENTS OF ACCUMULATIONS OF MAGNETIC PARTICLES



(57) Abstract: An apparatus is provided for quantitatively measuring groups of magnetic particles (11). The particles (11) are complexed with substances to be determined and are excited in a magnetic field. The magnetizations of the magnetic particles are thereby caused to oscillate at the excitation frequency in the manner of a dipole to create their own fields. These fields are inductively coupled to at least one sensor such as sensing coils (43) fabricated in a gradiometer configuration. The output signals from the sensing coils (43) are appropriately amplified and processed to provide useful output indications (67).

5
METHOD AND APPARATUS FOR MAKING MEASUREMENTS
OF ACCUMULATIONS OF MAGNETIC PARTICLES

10
Background of the Invention

Field of the Invention

15 This invention relates generally to sensing the presence of
magnetic particles, and more particularly to quantitatively
measuring accumulations of such particles by means of AC magnetic
excitation and inductive sensing of the amplitude of the
resulting oscillations of the magnetic moments of the particles
20 at the excitation frequency.

Discussion of Prior Art

25 Much attention has been given to techniques for determining
the presence, and possibly the level of concentration, of minute
particles in a larger mixture or solution in which the particles
reside. It is desirable in certain circumstances to measure very
low concentrations of certain organic compounds. In medicine,
for example, it is very useful to determine the concentration of

a given kind of molecule, usually in solution, which either exists naturally in physiological fluids (for example, blood or urine) or which has been introduced into the living system (for example, drugs or contaminants).

5 One broad approach used to detect the presence of a particular compound of interest, referred to as the analyte, is the immunoassay, in which detection of a given molecular species, referred to generally as the ligand, is accomplished through the use of a second molecular species, often called the antiligand,
10 or the receptor, which specifically binds to the first compound of interest. The presence of the ligand of interest is detected by measuring, or inferring, either directly or indirectly, the extent of binding of ligand to antiligand.

 A discussion of several detection and measurement methods
15 appears in U.S. Patent No. 4,537,861 (Elings et al.). That patent discloses several ways to accomplish homogenous immunoassays in a solution of a binding reaction between a ligand and an antiligand, which are typically an antigen and an antibody. Elings discloses creation of a spatial pattern formed
20 by a spatial array of separate regions of antiligand material and ligand material dispersed to interact with the spatial array of separate regions of antiligand material for producing a binding

reaction between the ligand and the antiligand in the spatial patterns and with the bound complexes labeled with a particular physical characteristic. After the labeled bound complexes have been accumulated in the spatial patterns, the equipment is
5 scanned to provide the desired immunoassay. The scanner may be based on fluorescence, optical density, light scattering, color and reflectance, among others.

The labeled bound complexes are accumulated on specially prepared surface segments according to Elings, or within an
10 optically transparent conduit or container by applying localized magnetic fields to the solution where the bound complexes incorporate magnetic carrier particles. The magnetic particles have a size range of 0.01 to 50 microns. Once the bound complexes are accumulated magnetically within the solution, the
15 scanning techniques previously described are employed.

Magnetic particles made from magnetite and inert matrix material have long been used in the field of biochemistry. They range in size from a few nanometers up to a few microns in diameter and may contain from 15% to 100% magnetite. They are
20 often described as superparamagnetic particles or, in the larger size range, as beads. The usual methodology is to coat the surface of the particles with some biologically active material

that causes them to bond strongly with specific microscopic objects or particles of interest (e.g., proteins, viruses, cells, DNA fragments). The particles then become "handles" by which the objects can be moved or immobilized using a magnetic gradient, usually provided by a strong permanent magnet. Thus, the Elings patent is an example of tagging using magnetic particles. Specially constructed fixtures using rare-earth magnets and iron pole pieces are commercially available for this purpose.

Although these magnetic particles have only been used in practice for moving or immobilizing the bound objects, some experimental work has been done on using the particles as tags for detecting the presence of the bound object. This tagging is usually done by radioactive, fluorescent, or phosphorescent molecules which are bound to the objects of interest. A magnetic tag, if detectable in sufficiently small amounts, would be very attractive because the other tagging techniques all have various important weaknesses. For example, radioactive methods present health and disposal problems. The methods are also relatively slow. Fluorescent or phosphorescent techniques are limited in their quantitative accuracy and dynamic range because emitted photons may be absorbed by other materials in the sample. See

Japanese Patent Publication 63-90765, published 21 Apr. 1988
(Fujiwara et al.).

Because the signal from a very tiny volume of magnetic particles is exceedingly small, it has been natural that
5 researchers have tried building detectors based on Superconducting Quantum Interference Devices ("SQUID"s). SQUID amplifiers are well known to be the most sensitive detectors of magnetic fields in many situations. There are several substantial difficulties with this approach, however. Since the
10 pickup loops of the SQUID must be maintained at cryogenic temperatures, the sample must be cooled to obtain a very close coupling to these loops. This procedure makes the measurements unacceptably tedious. The general complexity of SQUIDs and cryogenic components renders them generally unsuitable for use in
15 an inexpensive desktop instrument. Even a design based on so-called "high Tc" superconductors would not completely overcome these objections, and would introduce several new difficulties. See Fujiwara et al.

There have been more traditional approaches to detecting and
20 quantifying the magnetic particles. These have involved some form of force magnetometry in which the sample is placed in a strong magnetic gradient and the resulting force on the sample is

measured, typically by monitoring the apparent weight change of the sample as the gradient is changed. An example of this technique is shown in U.S. Patent Nos. 5,445,970 and 5,445,971 to Rohr. A more sophisticated technique measures the effect of the particle on the deflection or vibration of a micromachined cantilever. See Baselt et al., *A Biosensor based on Force Microscope Technology*, Naval Research Lab., J. Vac Science Tec. B., Vol 14, No.2 (pg. 5) (Apr. 1996). These approaches are all limited in that they rely on converting an intrinsically magnetic effect into a mechanical response. This response must then be distinguished from a large assortment of other mechanical effects such as vibration, viscosity, and buoyancy.

There would be important applications for an inexpensive, room-temperature, desktop instrument which could directly sense and quantify very small amounts of magnetic particles.

Summary of the Invention

Broadly speaking, the present invention provides a method and an apparatus for directly sensing and measuring very small accumulations of magnetically susceptible particles, e.g., magnetite, and consequently, their coupled substances of interest.

The magnetic particles or beads are coupled by known methods to analyte particles, thereby providing magnetic sample elements or magnetic bound complexes. A well-defined pattern of the magnetic sample elements is deposited on a surface on a holder.

5 The surface may be flat. A high-amplitude, high-frequency magnetic field is then applied to excite the particles in the sample. The field causes the particles to behave as a localized dipole oscillating at the excitation frequency. The fields from the sample are closely coupled to a sensor, such as an array of
10 inductive sensing coils, which may be fabricated in a gradiometer configuration. This configuration makes the sensing coils mostly insensitive to the large, uniform field that is used to excite the sample. Moreover, the geometry of the coils is designed to match the spatial pattern of the sample so as to provide a large
15 response that varies distinctively with the relative positions of the sample and coils.

The voltage induced across the sensor is carefully amplified and processed by phase-sensitive detection. An inductive pickup from the drive field itself may serve as the reference signal to
20 the phase detector circuit. The output of the phase detector is further filtered and digitized.

The signal amplitude is modulated by moving the sample with respect to the sensor. This allows the rejection of signals due solely to imbalance of the sensor, non-uniformity of the drive field, cross-talk in the circuitry, or any other source of apparent signal which is not due to the sample itself. The digitized shape of the signal amplitude with respect to the sample position is compared to the theoretical response shape using appropriate curve-fitting techniques, providing a very accurate estimate of the magnetic content of the sample in the face of inherent instrument noise and drift.

Brief Description of the Drawings

The object, advantages and features of this invention will be more clearly seen from the following detailed description, when read in conjunction with the accompanying drawings, in which:

Fig. 1 is a perspective view of a desktop version of an embodiment of the present invention;

Fig. 2 is an enlarged plan view of an embodiment of the sensor, showing sensing coils in the embodiment of Fig. 1;

Fig. 3 is a mechanical schematic perspective view of the embodiment of Fig. 1;

Fig. 4 is an electrical schematic diagram of the embodiment of Fig. 1;

Fig. 4A is an enlarged plan view of the substrate holding the sensing coils of Fig. 1;

5 Fig. 4B is a perspective view of a metal shield for the connection end of the substrate;

Fig. 5 is an enlarged plan view of an alternative embodiment of the sensing coils of the embodiment of Fig. 1;

10 Fig. 6 is a signal waveform of the output of the sensing coils versus the position of the magnetic material;

Fig. 7 is an embodiment of a lateral flow membrane sample holder which may be used in an embodiment of the present invention;

15 Fig. 8 is an E-core magnet system which may be used as the magnetic field source according to an embodiment of the invention (note that no drive coils are shown for clarity);

Fig. 9 is an embodiment of a microfluidic sample holder which may be used in an embodiment of the present invention; and

20 Fig. 10 is an embodiment of a single magnet pole piece with attached sensor which may be used in an embodiment of the present invention.

Detailed Description

Referring now to the drawing, and more particularly to Figs. 1 and 3 thereof, there is shown a preferred embodiment of the invention.

5

I. Reader Module

The reader module includes several distinct subsystems. These include: a sample holder with a motion control. The magnetic bound complex samples for measurement reside on the holder, and the same also provides the necessary relative motion within the system. A magnetizer or magnetic field source applies the excitation signals to the samples. Sensors, such as sensing coils, act as the signal pick-up for the signals generated in the samples. A drive circuit supplies the drive current to the coils of the magnetic field source. An amplifier/phase detector/digitizer is coupled to the sensor to receive and process the output signals therefrom. A microcomputer chip provides two-way communication between the external personal computer (PC) and the reader module.

20

A. Sample Motion Control

Magnetic particles are coupled to analyte or target particles by conventional methods to create magnetic bound complex samples. The analyte particles may include atoms, individual molecules and biological cells, among others. It is noted here that the terms "target particle" and "analyte particle" are used substantially interchangeably. It is further noted that the term "target" is not intended to be limited to the definition of that term as used in the field of DNA recombinant technology.

The magnetic bound complex samples are deposited in accumulations of several to several hundred particles at a number of predetermined positions 11 near the perimeter of a sample holder, such as disc 12 (Fig. 3). Other sample holders which may be substituted include lateral flow membranes, plastic strips, or holders employing lateral flow but without membranes. An embodiment employing lateral flow membranes is described in more detail below.

Another type of sample holder may employ microfluidics. A microfluidics system may have a sample sensing chamber and appropriate channeling to move a sample in or out of the sensing chamber using variations in pressure. For example, referring to

Fig. 9, a microfluidic system 151 is shown having an inlet channel 152. The inlet channel 152 is connected to a mixing chamber 164. A number of reagent chambers 154, 156, and 158 may be provided to hold various chemicals or reagents. As described below, they may also hold magnetically susceptible particles if desired. Near the periphery, or elsewhere, a sample analysis chamber 166 may be located. The location of this chamber is a predefined location and is where the sample magnetic measurement would occur. Accordingly, the sample holder must be configured to allow this chamber to be accessible to the sensor and the magnetic field source. Otherwise, the magnetic measurement may proceed as described elsewhere in this specification. Further processing may occur after the magnetic measurement. For this reason, a measurement chamber 168 is provided, which may also have its own reagent chamber 160. More reagent chambers may be provided if desired. An optional outlet or exit channel 162 may be provided. Such channels may not be necessary if the device is only a single-use device. Not shown in this figure for convenience but which may also be provided are various pressure inlets and valves which allow analyte particles, magnetically susceptible particles, and reagents to be shuttled around from chamber to chamber.

Analyte particles may be quantitatively measured via measuring their bound magnetically susceptible particles. In the microfluidic system, the samples may be introduced via the inlet channel as combinations of analyte and magnetically susceptible particles. Alternatively, the analyte particles may be introduced via the inlet channel and the two may be combined and mixed in the mixing chamber 164.

Variations of this system may be manyfold. For example, the sensor may be located directly on the microfluidic chip to match the region of analysis especially well. In another variation, a different parameter on the chip may be varied at the same time or at a different time, such as temperature. Temperature control means may be located on the chip or outside of the chip, such as in the case of laser heating within the mixing chamber. Such a system requires an optical window, as would be understood. Other parameters which may be varied may be anything that affects the presence or property of the magnetic tag, i.e., the magnetically susceptible particle, or its binding to the analyte particle.

The ways the bound complexes may be adhered to the pre-defined spots on the disc are known and may employ standard technology. The disc is mounted on an axial shaft 13 which extends downwardly to a toothed wheel 14. An appropriate

rotational device, such as a stepper motor 16, has a shaft 17 extending therefrom with a worm gear member 15 at the distal end thereof. The motor provides controlled rotary motion of disc 12 pursuant to signals applied from a PC 66 through a number of
5 wires 18. Of course, wireless coupling between the PC and the system of the invention could be used if desired.

In one preferred embodiment, as presently contemplated, disc 12 is about 47 mm in diameter and about 0.25 mm thick. It may be made of glass, plastic or silicon, for example. Its
10 thickness range, for practical functional purposes, would be about 0.1 mm to about 1.0 mm.

In the case where the sample holder is a lateral flow membrane, the sample holder may be made partially porous so that passage of the analyte particles through the porous portion of
15 the holder may be another parameter to be varied. In this case, the magnetically susceptible particles may be bound to the porous sample holder. For example, passage of the analyte particles through a porous portion of a holder may likely depend on the mass or size of the particles. Thus, the location of the
20 particles within the porous portion may be mass-dependent or size-dependent. As the analyte particles pass through the porous sample holder, they may bind preferentially and in a

predetermined manner to the bound magnetically susceptible particles. The bound samples, containing analyte particles combined with magnetically susceptible particles, may then be measured magnetically using the device embodied herein. The
5 porous portion of the holder may be replaced with, e.g., a filter as is known in the art. Such filters may be chosen to provide a suitable mass- or size- dependency according to the requirements of the process.

For example, referring to Fig. 7, a lateral flow membrane
10 101 is shown. Analyte particles may be flushed into a release pad 102 where they are released into a flow membrane 103. The particles may then flow by capillary action down the membrane and past a test line 106 on which bound magnetically susceptible particles are located. A control line 108 may also be provided.
15 Finally, an absorbent pad 104 may be located downstream if desired to collect the unbound analyte particles.

In operation, the test line may include colloidal iron particles coated with a material that specifically binds to a material in the analyte of interest. In this way, the test line
20 collects analyte particles preferentially. The control line 108 may have a known amount of colloidal iron for calibration or other such purposes. It should be clear that such a lateral flow

membrane may be replaced with, e.g., a gel electrophoresis test area. In this case, of course, the samples are not immobilized but may be moving past the sensing area.

5 The sample holder may also employ a reference device, such as a bar code, to provide a unique machine-readable tag to identify or locate an individual region or regions and the assay(s) that are associated thereby. The reference device may spatially index the location of an individual region or regions of analysis. The reference device gives a convenient way to
10 identify a sample of magnetic complex material. Besides bar codes, the reference device may alternatively employ a magnetic strip, a microchip, an optical reference, and so on. The reference device may be optically aligned with its corresponding sample for ease of reference.

15 The computer/CPU may read the reference information along with the magnetic (assay) signal and then display and store the assay results in the appropriate context. For example, an assay to measure the presence of e. coli would likely have results displayed in a different form than an assay testing for the
20 presence of binding of oligonucleotides. Since the substrate may be prepared specifically for each kind of assay, this information

can be encoded on the substrate as a bar code or using one of the techniques described above.

In this particular exemplary embodiment, motor 16 rotates wheel 14, which is connected to disc 12 by shaft 13, through a 120-tooth worm gear reduction. Of course, rotational drives having different particulars could also be employed.

A magnetic field source 21 may be moved linearly with respect to disc 12 by a rotational device, such as a stepper motor 22, having a 40 turn-per-circle lead screw 23 on a motor shaft 24. A boss 25 is configured with a hole having internal threads to which the spiral lead screw threads are coupled. The control signals are applied from microcomputer 65 to motor 22 through a number of wires 26. Again, the specifics of the rotational drive are set out here as an example only. Other appropriate elements having different characteristics could also be used.

For example, while the above system describes a situation where the magnetic field source is moved linearly with respect to the sample holder, another embodiment may be used in which the sample holder is moved relative to the magnetic field source. In this latter embodiment, the sample holder may be mounted to a shaft and mechanical drive system similar to the drive system

shown in Fig. 3. The drive system may move the sample holder into the gap of the magnetic field source in a controlled manner.

Numerous types of drive systems may be employed. These include stepper motors, screw and motor arrangements, hydraulics, magnetic drives, configurations in which a human operator physically moves the sample holder relative to the magnetic field source and relative to the sensor, pressure drives, pinch rollers, conveyor systems, etc.

The above describes the motion of the sample holder from a location in which samples may be loaded, such as on a disc, to a location near the magnetic field caused by the magnetic field source. Another motion that occurs in the system is the movement of the sample holder past the sensor. Various motions may be caused to accommodate this. For example, two-dimensional motion may be accommodated between the sensor and the sample holder. In the embodiment of Fig. 3, one degree of freedom motion (e.g., along an arc of a circle) is shown using motor 16. The drive system of motor 22 may also be employed to translate the sensor along another degree of freedom. Alternatively, another motor may be used to move the sample holder 12 along a similar degree of freedom. Finally, it should be noted that, by using

appropriate gearing, the same motor may be used to provide any combination of the above or different motions.

In other exemplary embodiments, the drive system may include a pinch roller which grasps a plastic strip on which a sample is disposed, moving the same past the sensor in a controlled fashion. Such an embodiment may be particularly useful where the sample is placed in a strip on a plastic card similar to a credit card, which is then "grabbed" by a device similar to that used in ATM machines. Of course, the drive system may also be any of the systems described above as well as other alternate systems.

B. Magnetic Field Source

Referring to Fig. 4, a ferrite toroid core 31, which is about 30 mm in diameter in the particular embodiment being described, is formed with a gap 32, which is about 1.5 mm wide. A drive coil 33 is wound as a single layer over about 270° of toroid 31, symmetric with respect to the gap. A feedback loop 34 encircles the toroid body at a location about 180° from (opposite) the gap. Loop 34 may be outside of coil 33 or between coil 33 and the toroid core. It may include a few or many turns, as necessary and appropriate for the feedback function. The purpose of the feedback loop is to sense or represent the field

in gap 32 and enable the signal processing or output circuit to self-correct for variations such as temperature drift. This loop is used to enhance precision and is not essential to proper operation of the system.

5 Various other magnetic field sources may also be used. For example, while most all employ electromagnets, the electromagnets may be in the form of, e.g., toroids or so-called "E-core"s which are magnets employing the shape of an "E" (see Fig. 8). In E-cores, the middle segment of the "E" is made somewhat shorter
10 than the outer segments. Referring to Fig. 8, two E-cores 112 and 112' are placed with their open sides facing each other. The shorter middle segments then define a small gap 114 therebetween. A sample on, e.g., a plastic strip 116 may then be situated in this small gap. The sensor used to measure the oscillation of
15 the magnetizations may be on a separate substrate 118 also located in the small gap or may alternatively be disposed on the end of one or both of the shorter middle segments. In any of the embodiments, in fact, the sensor may be disposed on a magnetic pole piece or other such element that forms a perimeter of the
20 gap. In this way, the unit may be made more modular and the coil placement more uniform and consistent.

In other embodiments, no gap is needed at all. Referring to Fig. 10, a single magnetic pole piece 201 may be situated with a sensor disposed thereon or disposed on a separate strip. In Fig. 10, the sensor is shown as two sensing coils 202 and 204. The pole piece can alternate the magnetic field, and the sensor can measure the oscillating magnetizations as above.

Referring back to Fig. 3, the toroidal magnetic field source assembly is mounted in insulative housing 35, which may be formed from fiberglass. Housing 35 has a slot 36 corresponding to the position of gap 32. This slot/gap is shaped and configured to selectively receive the edge of rotatable disc 12, and provides space for the sensing coil substrate, which is described in detail below.

15 C. Sensors

A sensor is used to measure the magnetic field strength of the samples. In this embodiment, the method used is AC susceptibility. A number of types of sensors may be employed. In the embodiments below, sensing coils connected in a gradiometer configuration are described. It should be noted that the gradiometer configuration is not necessarily required; moreover, other types of sensors may be used. These sensors may

include Hall sensors, GMR sensors, or other such sensors capable of measuring magnetic field strength or magnetic flux.

With particular reference now to Figs. 2, 4 and 4A, insulative substrate 41 is disposed in slot 36 in housing 35 and extends into gap 32. Bonding pads 40, 42 are provided at a proximal end of substrate 41 and a sensor, in particular sensing coils 43, is mounted adjacent a distal end of substrate 41. Preferably the substrate is made of sapphire or silicon and the sensing elements are thin film copper coils. Standard thin film fabrication techniques can be used to construct the substrate and sensing coils, where the leads to and from each coil are on separate different layers. For example, incoming traces 49 may be laid on the substrate surface by standard photolithographic processing methods, a layer of sputtered quartz may then cover the incoming leads, then coils 43 and output leads 44 are similarly applied and a protective layer of quartz may then be added on top. The usual means for connecting between the layers would be used.

The sensing coils, which are connected in series opposition creating a gradiometer configuration, are connected to bonding pads 40 and 42 by conductive traces 44 and 49, and thence to signal processing circuitry by twisted-pair wires 45. The
5 twisted pair arrangement is employed to assist in reducing stray signal or interference pickup.

In the spiral form shown in Fig. 2, the coil traces may be about 5 microns in width with about a 10-micron pitch between spiral traces. The thickness of the sensing coil traces may be
10 about 1 micron. The diameter of each completed coil is about 0.25 mm.

By making substrate 41 relatively long and narrow, bonding pads 40, 42 are relatively far away from the toroid gap, which helps minimize stray pickup in soldered leads 45. Metal
15 shield 46 (Fig. 4B) may be employed around the bonding area to further contribute to the reduction of stray signals or interference pickup. The shield is essentially a short piece of a thick-walled cylinder, typically formed of copper. The shield provides electrical shielding and facilitates mechanical
20 handling, but is not essential to operation of the embodiment of the invention. The connection (proximal) end of the substrate is slid into slot 50 after the wire connections are made.

An alternative embodiment of the sensing coils is shown in Fig. 5. The planar configuration of coils 47 is an elongated rectangle. The trace dimensions are about the same as for the Fig. 2 coils and the composite coil width is also about 0.25 mm.

5 The coil length is about 1-2 mm and the coils are connected to bonding pads 52, 53 by means of leads 48, 51.

In another alternative embodiment, two sets of coils may be used. One set of coils may be used as described above, to measure the magnetic moment of the sample. Another set of coils
10 may be employed within the same substrate as a reference set of coils. This reference set of coils may be disposed, e.g., on the side of the substrate opposite that of the sample set of coils. In any case, the reference set of coils is disposed far enough from the sample that the effect of the sample magnetic moment is
15 not detected by the reference set of coils. The reference set of coils is then used to measure the strength of the signal from an analysis region containing a predetermined amount of magnetic material or reference analyte. By comparison of the magnetic field detected by the sample set of coils with the magnetic field
20 detected by the reference set of coils, an even more accurate measurement of the sample magnetic moment may be made. To provide another reference, a magnetic standard may be employed as one of

the samples. When such a standard sample is measured, the results may be used to calibrate the system for future or previous measurements. This calibration may significantly help to reduce noise in the system. Auto-calibration may also be
5 employed with such a system, using the differential between signals, to zero the signal.

D. Drive Circuit

The magnetic drive circuit, shown at the left side of
10 Fig. 4, is built around a pair of high-current, high-speed operational amplifiers 54, 55. With the power provided by transformer primary winding 56, the amplifiers can provide in excess of about one ampere of drive current to magnetizing or drive coil 33 at about 200 kHz. This drive circuit is highly
15 balanced to minimize common-mode noise pickup in sensing loops or coils 43, 47.

Small secondary winding 57 coupled to loop 34 around the magnetizing coil provides a feedback voltage to operational amplifiers 54 and 55 to sustain oscillations at a well-regulated
20 amplitude and frequency. This secondary winding 57 also provides an optimum reference signal for the phase-detector circuitry, described below.

This embodiment describes an alternating field as the driving source for the complex of magnetic and analyte particles.

In a separate embodiment, the driving source may be non-sinusoidal, e.g., may be a field pulse or a square wave. A
5 variety of other such waveforms may also be used.

E. Amplifier/Phase Detector/Digitizer

A low-noise integrated instrumentation amplifier is the
10 basis for this circuitry, although somewhat better noise performance could be obtained using discrete components. Amplifier 61 is transformer coupled to the sensing coils in order to reduce common-mode noise signals and to facilitate a convenient way to null out imbalance in the magnetic field source
15 and in the sensor. The transformer coupling is conventional, is located in amplifier 61, and is not specifically shown in the drawing. In an alternative embodiment, amplifier 61 may be replaced by or supplemented with a preamplifier disposed on the substrate. In other words, substrate 41 may have patterned
20 thereon a preamplifier to modify the signals from the sensor prior to the phase-sensitive detection step. Phase sensitive detector 62 is also designed around a special purpose integrated

circuit. Phase sensitive detector 62 may be a phase-locking device or alternatively any other type of phase-sensitive device.

The output of the phase detector is applied to low-pass filter 63 and is then digitized in A/D converter 64. The

5 converter may be a high resolution, 20-bit sigma-delta converter, for example. Such a converter chip has adequate hum rejection at both 60 and 50 Hz, which proves to be very helpful in maximizing the sensitivity of the instrument. It is an off-the-shelf item, available from several manufacturers.

10

F. Microcomputer

Microcomputer 65 includes a microprocessor chip, such as a Motorola HC11, and has a built-in port which supports two-way serial communication to PC 66 by plugging into the serial port of

15 the PC. It also has specialized ports for communication with serial A/D converter 64 and stepper motors 16 and 22. A simple command language programmed directly into microcomputer 65 allows the PC to send commands and receive responses and data.

Microcomputer 65 may also perform many of the functions

20 previously described above. For example, microcomputer 65 may be equipped with a phase-sensitive device of its own, such as a

digital lock-in. Such a microcomputer 65 may acquire the signals, separate data from noise, and display the results.

G. Human Interface

5 The PC provides the operational command for the system. The PC runs the system through an RS-232 interface, e.g., from the microcomputer. The PC provides a display of the results of the measurements. The display may be, e.g., a computer monitor display or any other form of computer-assisted readout.

10

II. Operation of the System

In a relatively straightforward and known manner, a well-defined dot or pattern of the magnetic particle complexes comprising the samples is deposited on disc 12 at one or more
15 locations 11 near the periphery thereof. Pursuant to control signals from the PC, stepper motor 22 is energized to rotate lead screw 23 to move the magnetic field source assembly towards sample disc 12. When a sample position 11 near the peripheral
20 edge of disc 12 is aligned with a sensor such as sensing coils 43, 47 in the middle of toroidal gap 32, stepper motor 22 stops and a high amplitude (1 ampere, for example), high frequency (200 kHz) signal is applied to toroidal drive coil 33.

Again, while sensing coils are described below, it should be understood that a variety of sensors may be employed. A signal from PC 66 then energizes stepper motor 16 to rotate the disc and thereby move the sample dot past the sensing coils. The high
5 amplitude, high frequency magnetic field in gap 32 thereby excites the magnetic particles of the sample in the gap. The applied current is intended to drive the toroid to saturation, resulting in the field in the gap have a magnitude of about 1000 oersted. The particles then oscillate magnetically at the
10 excitation frequency, behaving as a localized dipole. Given the close physical proximity of the magnetic particles to the sensing coils, the magnetic fields from the sample are closely coupled to the gradiometer configured sensing coils. Because of the gradiometer configuration of the sensing coils, the output of the
15 sensing coils due to the large, uniform excitation field is substantially null or zero. In order to obtain the largest possible response, the geometry of the sensing coils is configured to match the spatial pattern of the samples. That is, the sample pattern dots are no larger than about 0.25 mm across.
20 The response signal varies distinctively with the relative position of the sample and the coils.

The signal from the sensing coils in the presence of the drive field and in the absence of a sample may serve as the reference signal to the signal processing portion of the system.

As the sample moves past one sensing coil and then the other,
5 the phase of the coil output signal reverses by 180° as shown in Fig. 6, thereby providing a very powerful detection technique. As shown in Fig. 6, the output may be shown as the response of the sensing coils versus the position of the sample with respect to the sensing coils. The induced voltage is amplified by
10 amplifier 61 and processed by phase detector 62. That signal is filtered and digitized and passed to the PC through microcomputer 65 to provide the output signals to the PC. Indicator 67 may be any type of useable device to provide information to the system operator. Indicator 67 could be a
15 visual indicator, conveying information numerically or graphically, or could also be a variety of lighting systems, audible indicators, or any combination of these or other possible indicators.

The output signal amplitude is modulated by moving the
20 sample with respect to the array of the sensing coils. This permits rejection of signals due solely to system and external inputs and not due to the sample itself. The digitized shape of

the signal amplitude with respect to sample position is compared to the theoretical response shape stored in PC 66 using appropriate curve fitting techniques. These techniques may include phase-sensitive techniques or other techniques yielding similar results. The result of this operation is a very accurate estimate of the magnetic content of the sample to the exclusion of inherent instrument noise and drift.

While a preferred embodiment of the invention has been presented above, some alternatives should be mentioned. Two sensor coil shapes have been shown but numerous other configurations may be employed. Moreover, as indicated above, sensors may be used which are patterned directly on one or more of the magnetic field source pole pieces. Furthermore, other varieties of sensors could be employed besides the types of coils disclosed. For example, balance hall sensors may be employed. In appropriate configurations, these may yield a frequency independent signal. Other sensors which may be advantageously employed include giant magnetoresistance (GMR) sensors, SQUID sensors, magneto-resistance sensors, etc.

In other variations, the magnetic field source is shown as moving with respect to the sample disc, but the disc and coupled stepper motor could be configured to move with respect to the

magnetic drive assembly if desired. The toroid core is shown with a rectangular cross section but other shapes are also feasible. As to the number of sample particles in a dot 11 on disc 12, by way of example, a 0.25 mm dot of sample elements
5 could contain about 10 five-micron size magnetic particles, or about 1200 one-micron size particles.

Thus, in view of the above description, it is possible that modifications and improvements may occur to those skilled in the applicable technical field which are within the spirit and scope
10 of the accompanying claims.

CLAIMS

What is claimed is:

1. An apparatus capable of quantitative magnetic measurement of sub-nanogram samples including combinations of analyte particles and magnetically susceptible particles, the samples arranged in defined patterns and disposed in a sample holder containing a plurality of samples, comprising:
 - a magnetic field source to apply an alternating magnetic field to the samples, the magnetic field source defining a gap in which a sample holder may be disposed;
 - a substantially flat magnetic field sensor to sense an induced magnetic moment from the samples and configured and arranged to substantially eliminate the contribution of the magnetic field source to the sensing, the magnetic field sensor having an output to communicate output signals, the magnetic field sensor disposed substantially within the gap of the magnetic field source; and
 - an electronic signal processor to process the output signals from the magnetic field sensor to provide a signal indicative of the quantity of the samples in the pattern.

2. The apparatus of claim 1, wherein the magnetic field sensor is one or more inductive sensing coils.

3. The apparatus of claim 2, wherein the inductive sensing
5 coils are connected in a gradiometer configuration.

4. The apparatus of claim 2, further comprising a set of reference coils spaced from said inductive sensing coils.

10 5. The apparatus of claim 2, wherein the inductive sensing coils are in the shape of circular spirals.

6. The apparatus of claim 2, wherein the sensing coils are rectangular in shape.

15

7. The apparatus of claim 1, further comprising a drive system, mechanically coupled to at least one of a sample holder or the sensor, wherein the drive system provides relative motion between the sample holder and the sensor.

20

8. The apparatus of claim 1, further comprising means for providing relative motion between a sample holder and the sensor.

9. The apparatus of claim 8, wherein said means is mechanically coupled to the sample holder or to the sensor.

5 10. The apparatus of claim 7, wherein the sensor is stationary and the drive system is structured and arranged to move the sample holder relative to the sensor.

11. The apparatus of claim 7, wherein the sample holder is
10 stationary and the drive system is structured and arranged to move the sensor relative to the sample holder.

12. The apparatus of claim 7, wherein the drive system includes a pinch roller mechanism.

15

13. The apparatus of claim 7, wherein the drive system includes:
a motor and screw arrangement for moving the sensor with
respect to the sample holder; and

a motor arrangement for moving the sample holder relative to
20 the magnetic field source.

14. The apparatus of claim 1, wherein the magnetic field source includes an electromagnet with a magnetically permeable core having an extended pole piece, and wherein the extended pole piece has the sensor mounted thereon.

5

15. The apparatus of claim 1, wherein the magnetic field source includes an electromagnet with a magnetically permeable core having a gap.

10 16. The apparatus of claim 15, wherein the sensor is patterned on the magnetically permeable core.

17. The apparatus of claim 15, wherein the magnetic field source comprises:

15 a toroid having a gap; and
 a drive coil wound around the toroid.

18. The apparatus of claim 17, wherein the sensor is patterned within the gap.

20

19. The apparatus of claim 15, wherein the magnetic field source comprises:

two E-core magnets wherein open ends of the E-core magnets are substantially facing each other and wherein at least one set of poles defines the gap therebetween; and

a drive coil wound around each E-core.

5

20. The apparatus of claim 19, wherein the sensor is patterned within the gap.

21. The apparatus of claim 15, further comprising a feedback
10 loop coupled to a field produced by the electromagnet, the output of the feedback loop connected to the signal processor, whereby the signal processor is capable of self-correcting for external influences.

15 22. The apparatus of claim 17, wherein the sensor is mounted on a substrate, the substrate and sensor capable of extending into the toroid gap.

23. The apparatus of claim 2, wherein the sensing coils number
20 two and are mounted on a substrate.

24. The apparatus of claim 1, wherein the signal processor comprises:

an amplifier coupled to the output of the sensor;

a phase sensitive detector connected to the amplifier to

5 condition the output signals;

an analog to digital converter to convert the output signals to digital form; and

a computer to receive the digitized signals and to provide control signals to the apparatus.

10

25. The apparatus of claim 1, wherein the signal processor comprises:

an amplifier coupled to the output of the sensor;

a computer in which is implemented within hardware or

15 software:

a phase sensitive detector connected to the amplifier

to condition the output signals;

an analog to digital converter to convert the output

signals to digital form; and

20 a controller to receive the digitized signals and to provide control signals to the apparatus.

26. The apparatus of claim 13, wherein:

the sample holder is a disc upon which a plurality of patterns of samples may be applied; and

the motor and screw arrangement includes a stepper motor
5 adapted to rotate the disc in a predetermined fashion.

27. The apparatus of claim 2, wherein the substrate is elongated

and has bonding pads on its proximal end to which conductors are connected to input and output signals from the sensing coils

10 which are mounted on the distal end of the substrate, the substrate further comprising a conductive shield around the bonding pads and the proximal end of the substrate to reduce stray signal and interference pickup.

15 28. The apparatus of claim 1, wherein the magnetic field source is configured and arranged to apply power as AC power.

29. The apparatus of claim 1, wherein the magnetic field source is configured and arranged to apply power in field pulses.

20

30. The apparatus of claim 1, wherein the magnetic field source is configured and arranged to apply power in square wave pulses.

31. The apparatus of claim 24, wherein the amplifier includes a circuit disposed on the substrate.

5 32. The apparatus of claim 1, wherein the sensor includes at least one Hall sensor.

33. The apparatus of claim 1, wherein the sensor includes at least one magnetoresistance sensor.

10

34. The apparatus of claim 33, wherein the sensor includes at least one giant magnetoresistance sensor.

15 35. The apparatus of claim 8, wherein the sample holder further comprises a reference device disposed thereon.

36. The apparatus of claim 35, wherein the reference device is a bar code.

20 37. The apparatus of claim 35, wherein the reference device is a magnetic strip.

38. A method for quantitatively measuring analyte particles using magnetically susceptible particles, comprising:

applying at least one sample pattern on a sample holder, the sample pattern including a plurality of bound complex particles,
5 each bound complex particle including an analyte particle combined with a magnetically susceptible particle;

creating a magnetic field;

exciting the magnetic particles in the pattern with the magnetic field to cause oscillations of the magnetizations
10 therein;

sensing the fields generated by the oscillating magnetizations; and

creating a signal representative of the sensed field.

15 39. The method as recited in claim 38, wherein the sensing includes sensing the fields using a pair of sensing coils connected in a gradiometer configuration.

40. The method as recited in claim 38, wherein the sample holder
20 is a rotatable disc.

41. The method as recited in claim 40, wherein the magnetic field is created in a gap in a toroidal core having a drive coil wound therearound.

5 42. The method as recited in claim 41, further comprising:
applying groups of sample patterns spaced around at least a
portion of the periphery of the disc;
moving the disc periphery into the gap in the toroidal core;
and
10 rotating the disc.

43. The method as recited in claim 38, wherein the magnetic field is created on a toroidal core having a drive coil wound therearound and the signal creating step is performed by a signal
15 processor, the method further comprising:
applying a drive signal to the drive coil to create the
magnetic field;
feeding back a signal representative of the drive signal in
the drive coil to the signal processor; and
20 correcting errors in the signal processor resulting from
external influences using the feedback signal.

44. The method of claim 43, wherein the drive signal is an AC signal.

45. The method of claim 43, wherein the drive signal is a field
5 pulse.

46. The method of claim 43, wherein the drive signal is a square wave.

10 47. The method of claim 38, wherein the signal creating includes displaying the representative signal versus the relative position of the sample pattern with respect to the location of the sensor.

15 48. The method of claim 38, further comprising sensing the magnetic field with a set of reference coils.

49. The method of claim 38, wherein the signal creating employs a phase-sensitive fitting technique.

20

50. The method of claim 38, further comprising:

applying at least one standard sample pattern on a sample holder;

creating a magnetic field;

5 exciting the magnetic particles in the standard sample pattern with the magnetic field to cause oscillations of the magnetizations therein;

sensing the fields generated by the oscillating magnetizations;

10 creating a signal representative of the sensed fields; and calibrating the sensor based on the signal created.

51. A computer program, residing on a computer-readable medium,
for quantitatively measuring analyte particles combined with
magnetically susceptible particles which form bound complex
samples, the computer program comprising instructions for causing
5 an apparatus to:
 create a magnetic field;
 excite the magnetically susceptible particles, that are
bound with analyte particles and which form bound complex
samples, and cause oscillations of the magnetizations therein;
10 sense the fields generated by the oscillating
magnetizations; and
 create a signal representative of the sensed fields.

52. An apparatus for quantitative measurement of analyte particles using magnetically susceptible particles, comprising:

a sample holder including:

an inlet for introduction of analyte particles;

5 a lateral flow membrane through which the analyte particles may flow, the lateral flow membrane including a predefined area containing a plurality of bound magnetically susceptible particles, whereby flowing analyte particles may become bound to the bound magnetically susceptible
10 particles;

a magnetic field source to apply an alternating magnetic field to the samples in the predefined area;

a magnetic field sensor having output signal conductors to communicate output signals; and

15 an electronic signal processor to convert the output signals from the sensor to provide a signal indicative of the quantity of the samples in the predefined area.

53. An apparatus for quantitative measurement of analyte particles using magnetically susceptible particles, comprising:

a microfluidic sample holder including:

an inlet channel for introduction of sample particles

including analyte particles; and

a sample analysis chamber connected to the inlet channel;

a magnetic field source to apply an alternating magnetic field to the samples in the sample analysis chamber;

a magnetic field sensor having output signal conductors to communicate output signals; and

an electronic signal processor to convert the output signals from the sensor to provide a signal indicative of the quantity of the samples in the sample analysis chamber.

54. The apparatus of claim 53, wherein the analyte particles are combined with the magnetically susceptible particles.

55. The apparatus of claim 53, further comprising:

at least one reagent chamber containing a solution of magnetically susceptible particles; and

at least one mixing chamber connected to the reagent chamber and to the inlet channel.

56. The apparatus of claim 53, further comprising a measurement
5 chamber connected to the sample analysis chamber via a channel.

57. The apparatus of claim 56, further comprising an outlet channel connected to one of the sample analysis chamber or the measurement chamber.

58. An apparatus capable of quantitative magnetic measurement of sub-nanogram samples, comprising:

5 a sample holder to contain a plurality of samples including patterns of combinations of analyte particles and magnetically susceptible particles;

a magnetic field source to apply an alternating magnetic field to the samples, the magnetic field source defining a gap in which the sample holder may be disposed;

10 a substantially flat magnetic field sensor to sense an induced magnetic moment from the samples and configured and arranged to substantially eliminate the contribution of the magnetic field source to the sensing, the magnetic field sensor having an output to communicate output signals, the magnetic
15 field sensor disposed substantially within the gap of the magnetic field source; and

an electronic signal processor to process the output signals from the magnetic field sensor to provide a signal indicative of the quantity of the samples in the pattern.

59. An apparatus capable of quantitative magnetic measurement of sub-nanogram samples comprising:

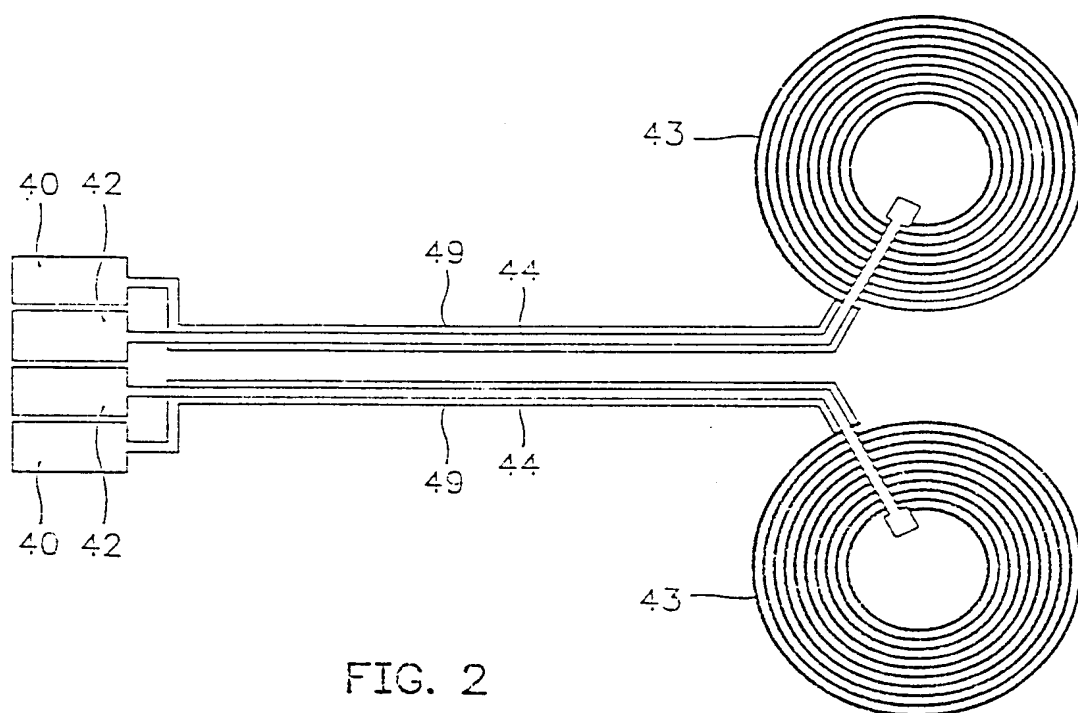
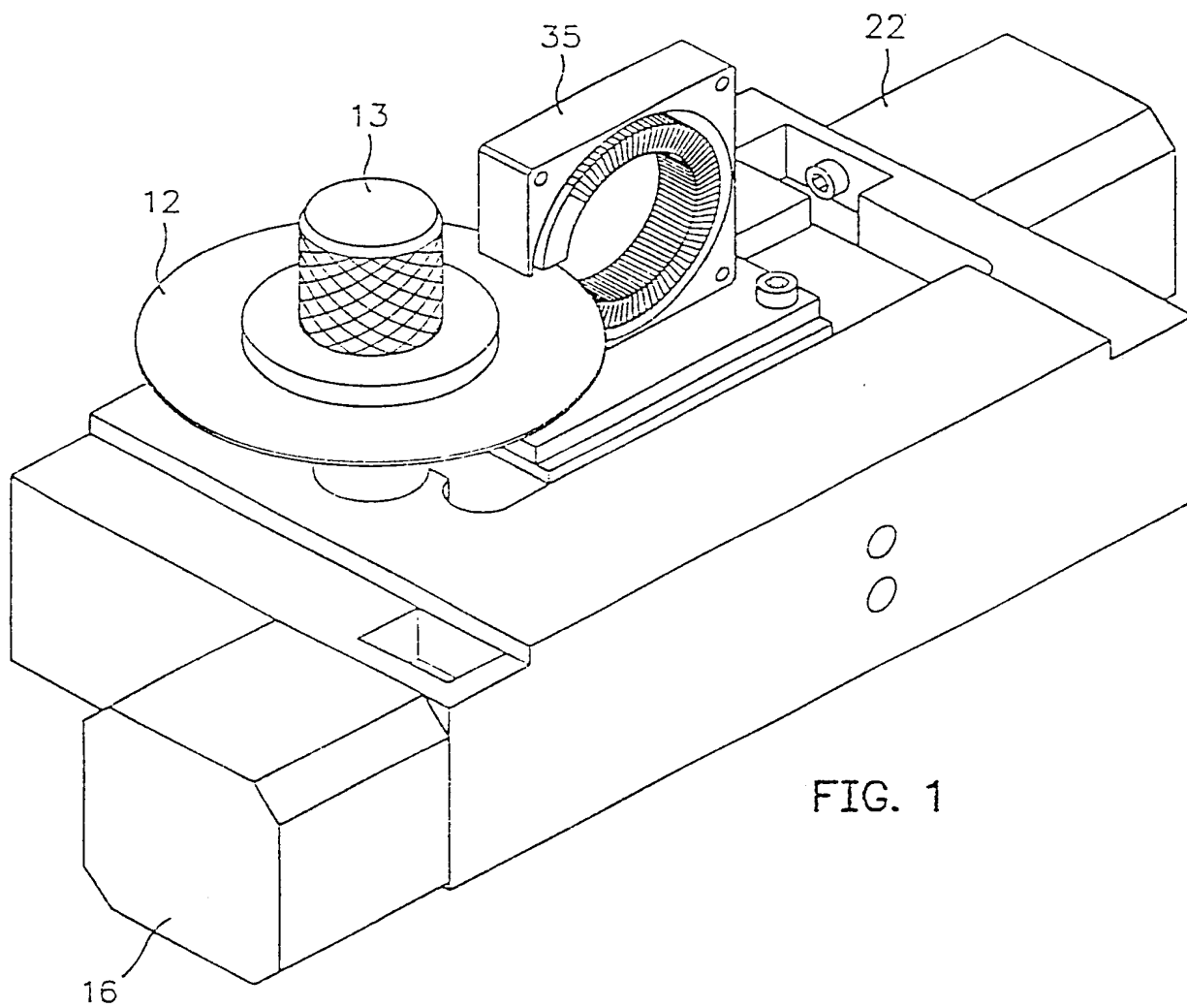
a sample holder to contain an extent of a plurality of samples including combinations of analyte particles and

5 magnetically susceptible particles;

a magnetic field source to apply an alternating magnetic field to the samples, the magnetic field source defining a gap in which a sample holder may be disposed, the gap having a width of less than about 5 mm;

10 a substantially flat magnetic field sensor to sense an induced magnetic moment from the samples and configured and arranged to substantially eliminate the contribution of the magnetic field source to the sensing, the magnetic field sensor having an output to communicate output signals, the magnetic
15 field sensor disposed substantially within the gap of the magnetic field source and having a sensing area substantially the same as the extent of the plurality of samples and having a dimension of the sensing area greater than a spacing between the plurality of samples and the magnetic field sensor; and

20 an electronic signal processor to process the output signals from the magnetic field sensor to provide a signal indicative of the quantity of the samples in the pattern.



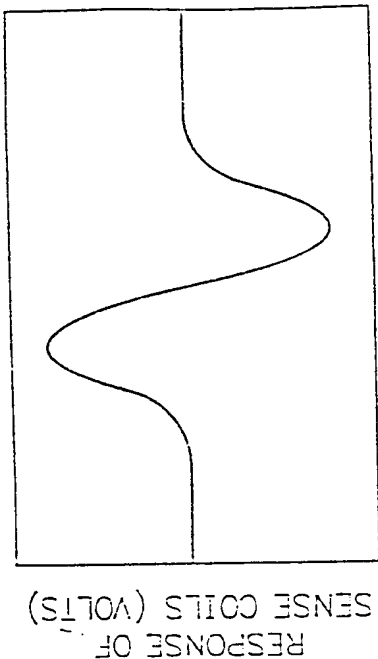


FIG. 6

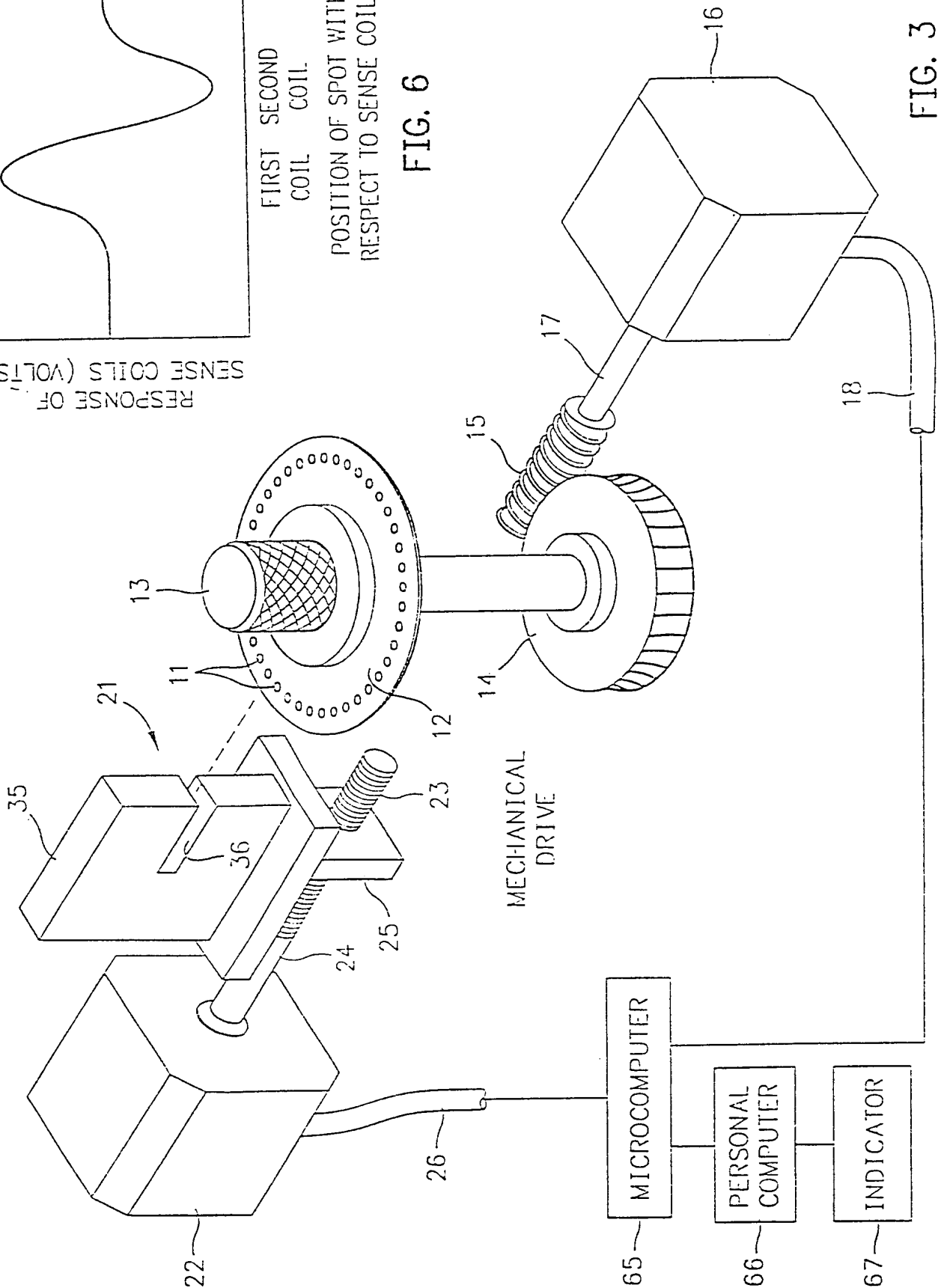


FIG. 3

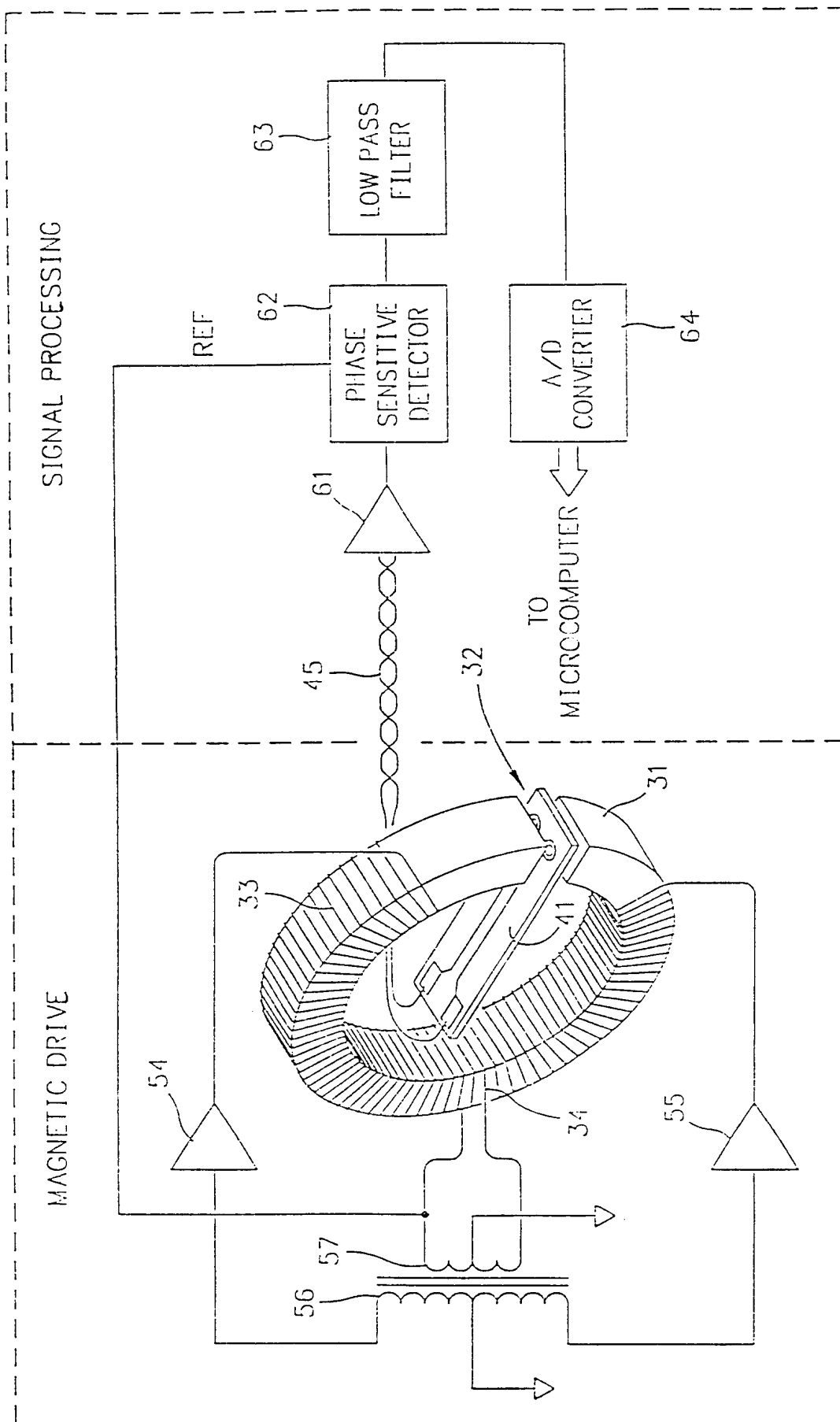


FIG. 4

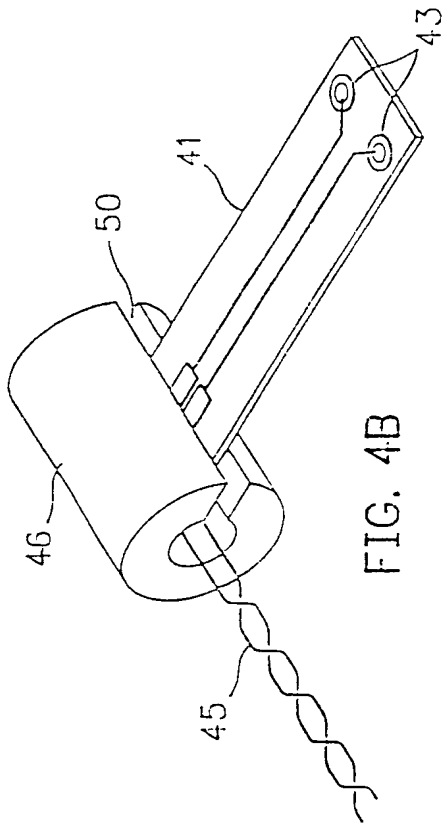


FIG. 4A

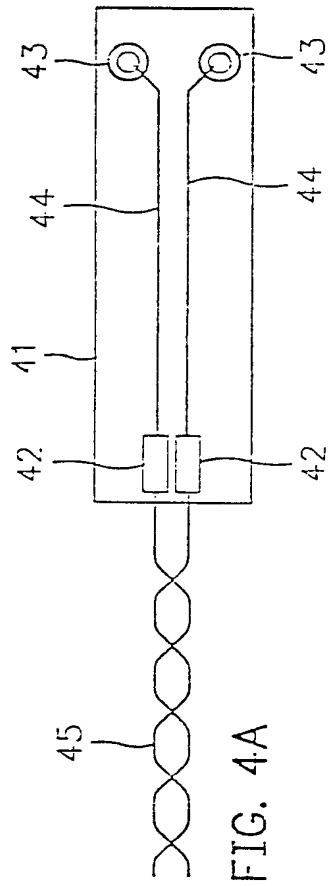


FIG. 4B

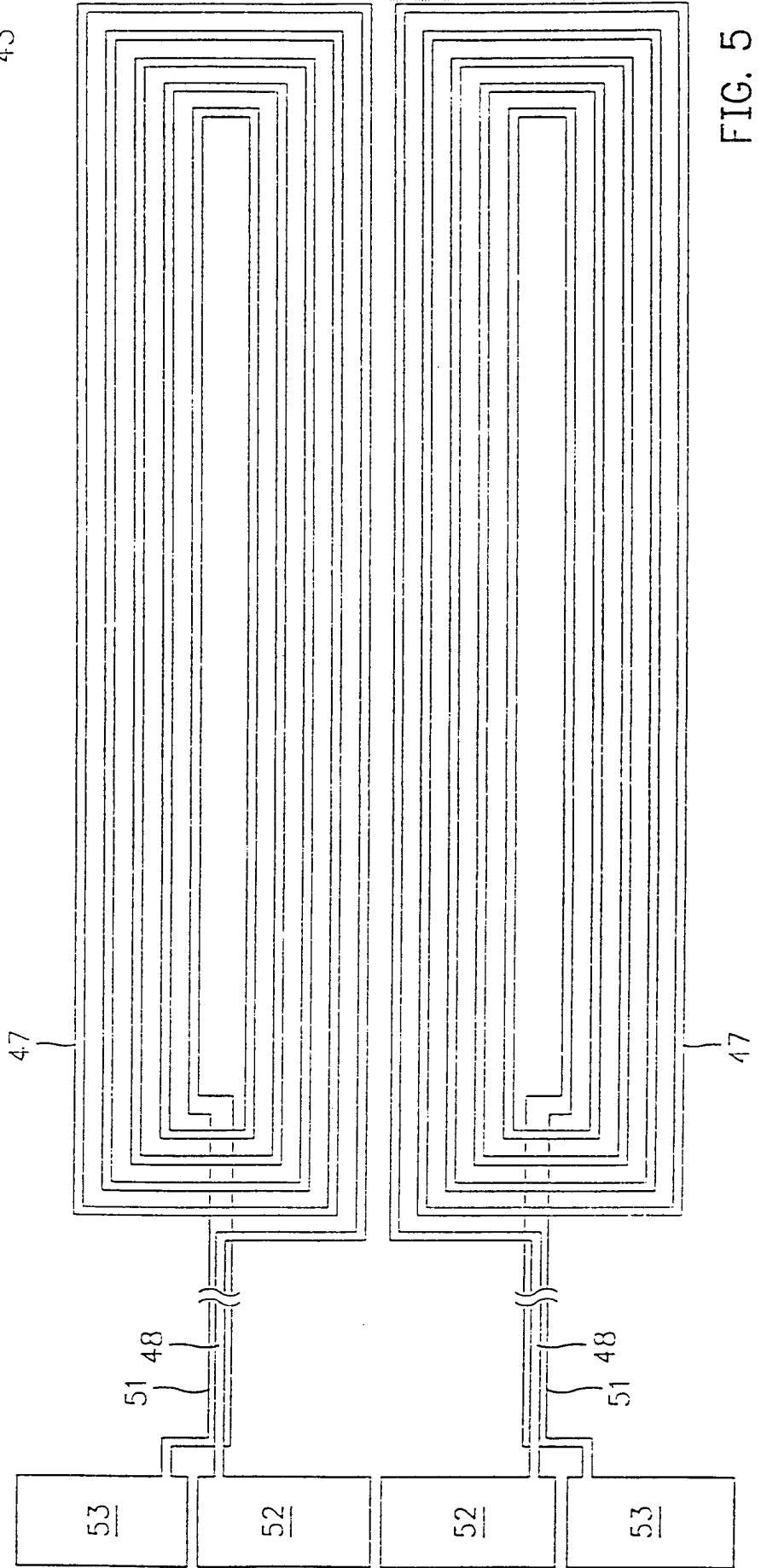


FIG. 5

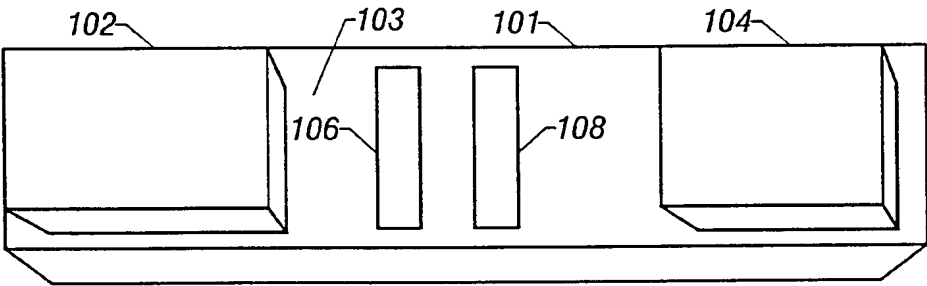


FIG. 7

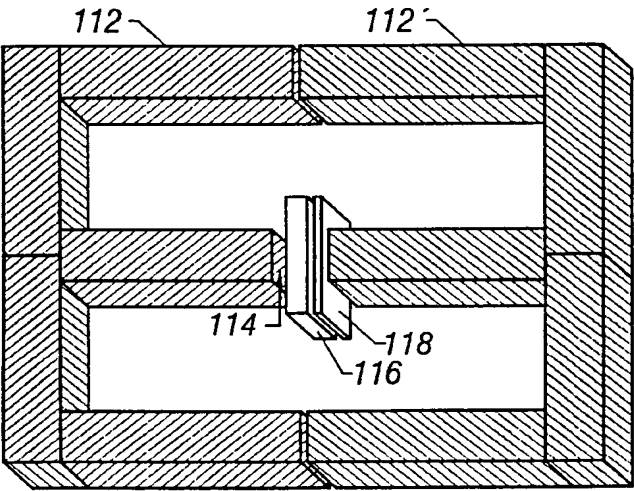
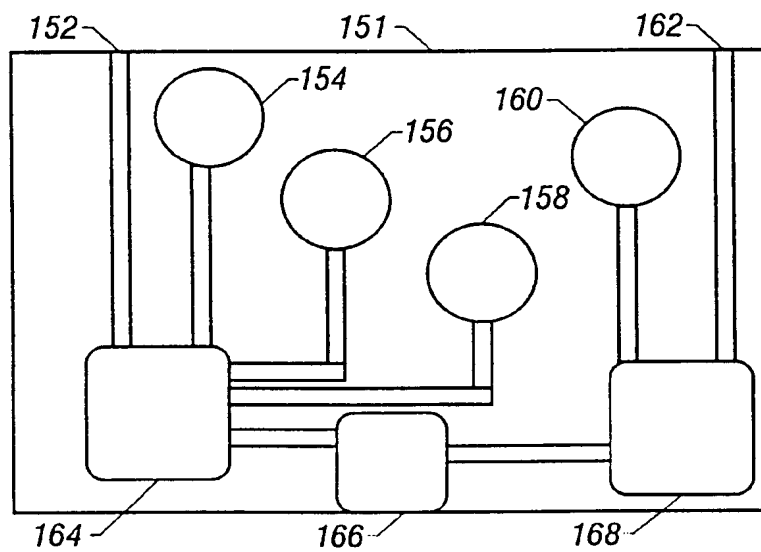
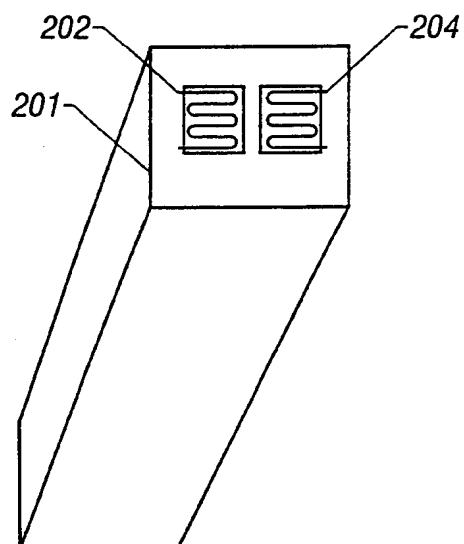


FIG. 8

**FIG. 9****FIG. 10**

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/42353

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :G01N 27/72

US CL :324/239

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 324/239, 71.1, 71.4, 201, 204, 225, 226, 232-234, 236, 243; 422/68.1; 436/526

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

NONE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

USPTO APS EAST

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5,034,689 A (INOUE et al) 23 July 1991 (23.07.1991), see whole document.	1-59
A	US 5,001,424 A (KELLETT et al) 19 March 1991 (19.03.1991), see whole document.	1-59
A	US 5,486,457 A (BUTLER et al) 23 January 1996 (23.01.1996), see whole document.	1-59

☐ Further documents are listed in the continuation of Box C.

☐ See patent family annex.

* Special categories of cited documents:	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A" document defining the general state of the art which is not considered to be of particular relevance	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
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"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&" document member of the same patent family
"O" document referring to an oral disclosure, use, exhibition or other means	
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

16 MARCH 2001

Date of mailing of the international search report

05 APR 2001

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